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THE REPRODUCTIVE CYCLE OF *SPHAERIUM SIMILE* [1]

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Reproduction in the fresh water family Sphaeriidae shows a number of consistent and specialized characteristics. Virtually all members of the family are hermaphroditic, and the developing young are retained in special brood pouches known as marsupial sacs, located in the inner gill of the adult, until fully prepared for a free living existence. The general structure, histology and development of the marsupial sacs, particularly in the genus *Sphaerium*, have been extensively studied (Poyarkoff, 1910; Groenewegen, 1926; Okada, 1935b). Other investigators have made observations on a number of different aspects of reproduction in the sphaeriids. Drew (1896) described the general anatomy of the genital system of *Sphaerium sulcatum* (= *simile*; Herrington, 1965). Gilmore (1917) gave a brief outline of some of the main points of reproduction in *Sphaerium simile*. Woods (1931) described the development of the genital system in *S. striatinum* from the time of fertilization through the attainment of sexual maturity in the individual. Foster (1932), by analyzing seasonal changes in the relative abundance of individuals of different sizes in the population, determined the life cycle of *S. solidulum* (= *striatinum*; Herrington, 1962). Okada undertook a very thorough investigation of virtually every aspect of reproduction in *S. japonicum* (formerly identified as *Musculium heterodon*). In a series of 4 papers (1935a, 1935b, 1935c, and 1936, he described the genital system, gametogenesis, the structure, development and function of the marsupial sacs, the reproductive cycle, and the early embryology of the species.

More recently, Thomas (1959, 1963, 1965) studied the growth and life history of a population of *Musculium partumeium* living in a temporary pond. It is evident from these studies that the life cycle of some members of the family Sphaeriidae may be particularly suited to meet unusual environmental conditions. Heard (1965) compared the life cycles of a number of species of the genus *Pisidium* found in North America. Another recent work involved a general examination by Mitropol'skii (1969) of the life cycle of *S. corneum*, a species previously studied by Thiel from 1924 to 1930 (cited in Okada, 1935b). Finally, Avolizi (1971) has investigated growth rate, mortality, fecundity and biomass turnover in populations of *S. simile* and *S. striatinum* located in New York State.

It would appear from the number and scope of the investigations already undertaken that virtually no stone has been left unturned in the examination of reproduction in the Sphaeriidae. Heard (1965), however, noted that there are marked differences in reproductive cycles among the several genera of this family. These differences include the relative life span of the species, the size and number of

[1] This paper represents an abridgement of a thesis submitted to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Master of Science.

litters produced each year, and the exact seasons in which such processes as gametogenesis are most active. Furthermore, he noted that differences also occur among different species of the same genus and occasionally among members of the same species found in different environments.

Heard's observations have made it quite evident that many aspects of reproduction in the genus *Pisidium* are highly variable. A review of the literature in the field clearly indicates that as in *Pisidium,* seasonal variations in the time of gametogenesis, fertilization and birth occur among species of *Sphaerium* as well, and many significant details of reproductive biology remain to be elucidated, particularly in species such as *Sphaerium simile* which have not been exhaustively studied.

Although Drew (1896) described the genital system of *Sphaerium simile* and Gilmore (1917) studied some of the major aspects of its reproductive morphology and physiology, no one has undertaken a detailed investigation of the reproductive cycle of this species. The purpose of the present study, therefore, was to elucidate the annual reproductive cycle, for comparative purposes, in a species in which it has not been examined.

H. B. Herrington, an authority on the sphaeriids, has made a number of revisions in the taxonomy of this group which will be used in this paper (Herrington, 1962 and 1965). He indicates that the name *Sphaerium striatinum* should replace *Sphaerium solidulum* (Herrington, 1962), and the species identified as *Sphaerium sulcatum* should now be referred to as *Sphaerium simile* (Herrington, 1965). In addition, the clam identified by Thomas (1959, 1963, and 1965) as *Sphaerium partumeium* is properly termed *Musculium partumeium* (Herrington, 1965).

Materials and Methods

A large population of *Sphaerium simile* occupies the soft, flocculent, silty bottom of the stream draining the large pond in the Lloyd-Cornell Reservation, locally referred to as McLean Bog, located off Route 13 between Dryden and Cortland, New York. This population is such a conspicuous feature that the stream is known as Sphaerium Brook (Sibley and Leffingwell, 1926). Information concerning the environmental characteristics of this brook and their probable relationship to the reproductive cycle of this population of *S. simile* will be cited in the discussion.

Collections were made in the brook on about the twentieth of each month from November, 1969 through October, 1970. Because of inclement weather in February, 1970, it was impossible to obtain a collection for this month until March 2; the normal March collection was taken on March 21. Specimens were obtained by scooping up the substratum several times with an ordinary 6 to 8 inch kitchen sieve, mesh size about $\frac{1}{16}$ of an inch, with the objective of securing a random sample consisting of varying numbers of individuals. The number of specimens collected each month ranged generally between 100 and 300. The material was suspended in water from the brook for transportation to the laboratory.

At the time of each collection a record was made of the water temperature in the brook, which was found to range from 2° C in December, 1969 to 26° C in August, 1970

At the laboratory the clams were immediately sorted from the substratum and placed in large fingerbowls containing aerated brook water, set in a water bath maintained at about 15° C. From each collection, 15 individuals ranging in size from 8.5 or 9 mm to over 16 mm were selected for histological examination. The same numbers of specimens of exactly the same sizes were not chosen each month, but an overall effort was made to select approximately equal numbers of clams of various sizes. Only those animals that had their feet and siphons extended and otherwise appeared to be in good condition were chosen. These individuals were measured with vernier calipers or a millimeter scale placed under a dissecting microscope. They were then thoroughly relaxed in approximately 300 ml of brook water containing several drops of Propylene Phenoxetol (Owen, 1955). This compound was obtained from Goldschmidt Chemical Corp., New York, New York.

When the valves were gaping widely, usually after about an hour in the relaxing solution, each clam was fixed in warm Bouin's fluid. It was allowed to remain in the fixative for several weeks, until the shells of the embryos it contained were completely decalcified. At this time the remains of the adult valves were removed with fine forceps. The soft tissues were then soaked for a few days in several changes of 70% ethyl alcohol, dehydrated, and embedded in paraffin in a vacuum oven. Serial cross sections of the entire area between the anterior and posterior adductor muscles were cut at 8 μ to 10 μ and mounted on albuminized slides. The sections were stained in Delafield's or Ehrlich's hematoxylin and counterstained with a 0.1% solution of eosin Y in 95% alcohol.

After the specimens selected for histological preparation had been fixed, the lengths of all the remaining individuals in the sample were measured with vernier calipers or a millimeter scale and the number of clams in each size category (taken at 1 mm intervals) recorded. These data were used to calculate the percentage of "newborn" young in each monthly sample.

Each month from January through October 1970, approximately 20 specimens, ranging in length from 13.5 mm to over 16 mm, were dissected, and the embryos of maximum size that they contained were removed and measured. Data recorded included the length of the parent and the length of the largest embryo in each of its inner gills. Despite the fact that only the maximum size embryos in the parents were measured, these young showed considerable variations in length each month, sometimes ranging from less than 1 mm to approximately 7 mm long. These measurements were made, at the latest, on the day after the collection had been made. Only a few such measurements were recorded for specimens collected in November and December, 1969.

Observations

Seasonal changes in the testes

As previously described by Drew (1896) and Gilmore (1917), the male reproductive system of *Sphaerium simile*, which is located behind the stomach and below the pericardial cavity, consists of paired gonads, each composed of a number of testicular follicles grouped around a common sperm duct. Although the 2 gonads are at first separated by the intestine, in the more posterior regions of the animal this barrier disappears and the groups of follicles come to lie close together above the foot, a condition that frequently obscures the paired nature of the gonads at this point. It is at this level that the gonadal mass reaches its greatest width, and data to be cited later refer to the breadth of the organ at this point.

The overall condition of the male gonads in terms of their size and activity varies both with the seasons and with the size of the animal, and these changes will now be described. The exact size at which *Sphaerium simile* reaches sexual maturity is not known, since in even the smallest individuals examined (*i.e.*, approximately 8.5 mm) the testes contain spermatozoa.

In animals collected from September through April the male gonads show many consistent characteristics. In specimens less than 12 mm in length, the entire gonadal mass usually ranges from 0.2 mm to 0.45 mm in greatest width, although in some cases this measurement may exceed 0.5 mm. In the majority of larger individuals (*i.e.*, exceeding 12 mm in length), the entire gonadal area varies from 0.3 mm to 0.7 mm in greatest width, but usually it is approximately 0.5 mm to 0.6 mm wide.

During this period specimens ranging from 8.5 mm to just below 12 mm in length appear to be undergoing moderate amounts of spermatogenic activity (Figs. 1 and 3). Spermatogonia and to a lesser extent spermatocytes are readily visible, particularly in the early spring (especially April) when they frequently fill the entire follicular cavity, obscuring any evidence of a central lumen (Fig. 3). In many follicles, however, particularly in those found in smaller individuals collected before April, cells in more advanced spermatogenic stages are often located in the interior regions of the follicle. Spermatids can often be seen in the fall, although they are considerably less common from December through April. In many testicular follicles there is a distinct central lumen which contains spermatozoa, although this phenomenon is not as common in April as it is in previous months. Eosinophilic material (to be described in more detail below), although occasionally visible in specimens over 10 mm in length, is mostly found in small amorphous aggregations, usually confined to the periphery of the follicle.

In the majority of specimens over 12 mm in length collected between September and April, the testicular follicles show evidence of a decided reduction in spermatogenesis. The gonads may contain one or a few follicles which have retained spermatogenic activity, but the majority of follicles contain a mixture of spermatogenic cells and eosinophilic material (Fig. 2). The latter substance, although occurring in a variety of shapes, is often arranged in prominent spheres, many of which exceed 20 μ–25 μ in diameter. With the stains employed, its color varies from deep pink to purple. When the eosinophilic bodies are the predominant material in the follicles, as frequently happens, the spermatogenic cells are fewer in number, show no evidence of the organization of cell types that is normally characteristic of spermatogenesis and are considerably reduced in variety. In particular, spermatids and spermatozoa are rare, especially from December until April. In extreme cases the eosinophilic bodies are the only material found in the follicle.

Eosinophilic material is never found to any great extent in follicles in which active spermatogenesis is occurring. Although blood cells have not been seen in the process of phagocytosis, they are sometimes found adhering to the outer wall of a testicular follicle or, more rarely, actually within a follicle or sperm duct.

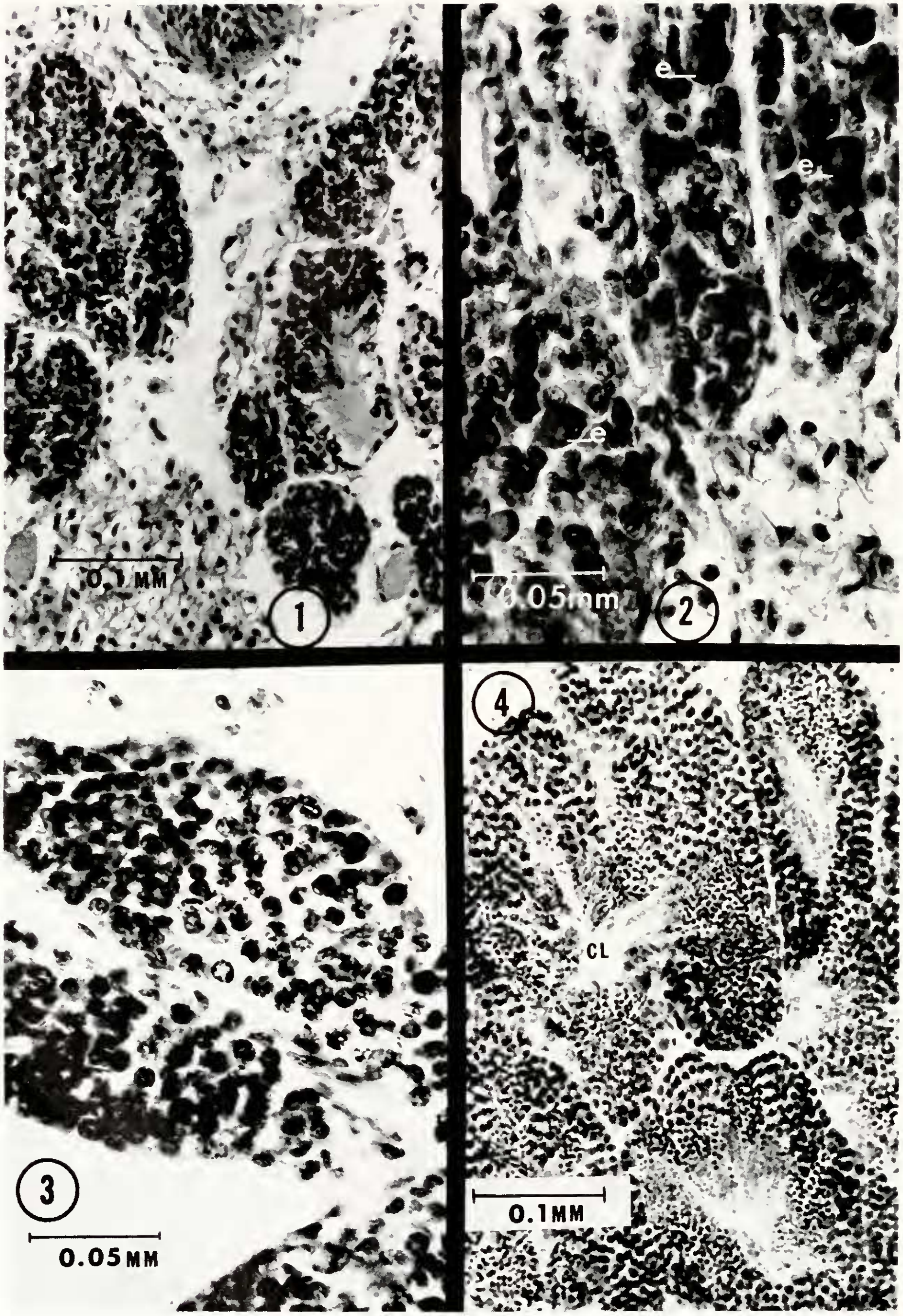

FIGURE 1. Testicular follicles of specimen 10 mm in length collected in December. In December (as in the other months from September to April) specimens under 12 mm in

Occasionally rounded masses of sperm heads can be seen which are apparently surrounded by an eosinophilic substance. In general, the evidence suggests that the eosinophilic material may represent the results of the phagocytosis, by blood cells, of debris and degenerating follicular contents.

With few exceptions, as indicated above, by April the testes of the average specimen show very few spermatozoa. This situation results from the fact that while the smaller specimens have gonads with little sign of degeneration, the follicles contain chiefly immature spermatogenic cells. The larger specimens, on the other hand, are showing extensive testicular degeneration and decreasing spermatogenic activity.

In May specimens over 12 mm in length may contain at least a few follicles whose poor development, obvious shrinkage and large quantities of eosinophilic material are reminiscent of conditions found in preceding months. Nevertheless, from May to July the great majority of individuals examined show a marked increase both in the overall size of the gonads and in the amount of spermatogenic activity taking place. The contrast between the gonads of smaller and larger individuals is much less pronounced than during the period from September through April.

From May through July, specimens under 12 mm in length have testes ranging from 0.5 mm to 0.75 or 0.8 mm wide. In specimens longer than 12 mm the overall range in maximum width of the gonad is from 0.5 mm to well over 1.0 mm, but most commonly the gonadal mass in these individuals is approximately 0.7 mm to 0.8 mm wide.

The testicular follicles contain abundant quantities of cells in all stages of spermatogenesis (Fig. 4). Spermatids show a particularly marked increase from previous months. They often occupy much of the interior of the follicle, and especially during June and July, frequently extend out to the periphery as well. Although spermatozoa are visible in May, they are far less numerous than other cell types. In June and July, on the other hand, spermatozoa are usually readily visible in varying numbers, frequently filling a central lumen which may be quite prominent (Fig. 4). Even in June and July, however, the younger stages still greatly outnumber the spermatozoa, indicating an obviously great potential for further production of male gametes.

It has already been indicated that in May specimens over 12 mm in length

length show evidence of moderate spermatogenic activity and little sign of testicular degeneration.

FIGURE 2. Testiclar follicles of specimen 13 mm in length collected in December. Testicular follicles such as those illustrated in this figure are characteristic of specimens longer than 12 mm collected from September through April. They are also commonly found in adults greater than 12 mm in length collected in August. Note the prominent eosinophilic masses mingled among the spermatogenic cells; e, eosinophilic mass.

FIGURE 3. Testicular follicles of specimen 11.3 mm in length collected in April. Particularly in April, the testicular follicles of clams less than 12 mm long often have spermatogonia and spermatocytes extending throughout the entire follicular cavity, obscuring any evidence of a central lumen.

FIGURE 4. Testicular follicles of specimen 12.7 mm in length collected in June. In June the testicular follicles of most specimens, regardless of size, are quite massive and show evidence of very active spermatogenesis. The prominent central lumen is lined with abundant spermatozoa; CL, central lumen.

FIGURE 5. Ovaries. Note the characteristic manner in which developing oögonia bulge into the ovarian lumen. The spherical cell completely free in the lumen (arrow) is a primary oöcyte.

often contain at least a few testicular follicles with large quantities of eosinophilic material. In general, however, from May to July this substance, although occasionally visible, is not common in the testes of the majority of individuals examined, regardless of size.

There is evidence of declining spermatogenic activity in some of the specimens longer than 15 mm found in July as well as in the majority of specimens greater than 12 mm in length collected in August. In general the testes are similar in their overall size and follicular contents to those of clams over 12 mm long examined from September to April. The testes of these specimens often contain much more eosinophilic material at the expense of spermatogenic cells, particularly spermatozoa. Nevertheless, cells in all stages of spermatogenesis are still readily visible and are definitely in the majority in most of the follicles examined.

In August, the testes of specimens less than 12 mm in length range from approximately 0.2 mm to 0.45 mm in greatest width. Despite this decrease in size, however, the testes show evidence of active spermatogenesis, and all cell types can be found in varying proportions in the testicular follicles. In addition, eosinophilic material is generally not common in individuals less than 12 mm long.

Seasonal changes in the ovaries

In *Sphaerium simile* as in other members of the genus *Sphaerium*, the paired ovaries are located behind the testes and above the foot. Each ovary tapers posteriorly and merges with a hermaphroditic duct which also receives the products of the sperm duct.

The general process by which mature female gametes are produced in *S. simile* is virtually identical to the detailed and precise account of oögenesis given by Okada (1935a) for *S. japonicum*. In both species cells of the ovarian epithelium develop into oögonia, gradually accumulate cytoplasm and bulge into the ovarian lumen until their bases of attachment to the ovarian wall become extremely tenuous. Eventually the cells are released into the lumen as primary oöcytes. Cells in various stages of oögenesis are illustrated in Figure 5. In *S. japonicum* the maturation divisions of the egg follow soon after sperm penetration. In the present study these events have not been observed, but it seems safe to assume that they occur in a similar manner in *S. simile*.

Oögenesis occurs throughout the year in all clams examined, although there are variations in the extent of this activity among individuals which are not firmly correlated with seasonal differences or with the size of the specimen. Within a single individual, in addition to very early oögonial stages, older oögonia of varying sizes can be found clearly bulging into the ovarian lumen (Fig. 5). Most commonly these developing cells extend to a distance of 25 μ to 46 μ into the cavity, but in those adults in which oögenesis is at a low level, most of the prominent oögonia

FIGURE 6. Several very early fetal larvae in a marsupial sac (one of the embryos is indicated by an arrow). Note that these embryos are primitive in development and bear no resemblance to typical adult clams.

FIGURE 7. Embryo in the earliest stage of "intermediate" development. In this section, mantle buds and a definite foot are readily visible, giving the embryo a more "clam-like" appearance than that of previous embryonic stages; MB, mantle bud; F, foot.

FIGURE 8. Very large embryos (indicated by arrows) lying in each inner gill of the parent clam. These young are over 5 mm in length, well developed, and nearly ready to be born.

are approximately 25 μ to 35 μ in height. In cases where the level of activity is greater, a phenomenon most commonly found in specimens collected from June through August, many of the cells are larger, extending 35 μ to 46 μ, and sometimes as much as 60 μ into the lumen. In addition, at least some of these cells show evidence of a decidedly narrowing base of attachment to the ovarian wall. Normally each adult has from 10 to well over 30 oögonia clearly extending into the ovarian lumen. Although smaller individuals tend to contain fewer such cells than larger adults, there is no definite relationship between the size of the specimen and the number of oögonia it contains.

Primary oöcytes (*i.e.*, cells ranging from approximately 35 μ × 28 μ to 58 μ × 46 μ lying free in the ovarian lumen) are considerably less numerous than the earlier stages of oögenesis. Generally, at least one adult in every monthly collection contains one or a few, and these cells are more readily found in specimens collected during the summer. Primary oöcytes are particularly common in June, when over 40% of the individuals examined have ovaries containing well developed cells unmistakably free in the ovarian cavity. Throughout the year, some individuals can also be found containing prominent cells which seem to be lying against the wall of the ovary with no obvious attachment to it. These cells do not clearly appear to be free in the lumen, but even if they have not yet been released, their appearance and relation to the ovarian epithelium make it seem obvious that they will soon be primary oöcytes.

The general pattern suggested by these observations shows a continuous low level of egg production throughout the year, with a recognizable peak occurring in June. In addition, it appears that oögenesis is generally more active during the summer than in other seasons. The fact that oögonia tend to be larger at this time, and the fact that primary oöcytes are somewhat more common, lend support to this view.

Sphaerium simile must be at least 9 mm long and more often over 10 mm in length before it begins to brood young. The younger adults in the population undergo several periods of fertilization while they themselves are increasing in length. By this process they gradually accumulate young in several stages of development. By the time they are 15 mm in length, adults are usually maintaining the maximum number of young customarily found in a member of this species. Clams of this size usually contain at least 6 to 8 marsupial sacs, and the young within the parent are in 3 or 4 different phases of development. Normally a marsupial sac contains only one embryo, but 3 or 4 young may be enclosed in one brood pouch if they are in very early developmental stages (*i.e.*, zygotes to early "fetal larvae"). Only individuals at least 13.5 mm in length contain embryos ready to be born, and even in animals of this size the phenomenon is not common. Embryos very near birth are most frequently found in adults from 15 mm to 17 mm in length.

The number of embryos in a parent of specified length shows little seasonal variation, but the developmental stages of young that are present may differ in consistent ways depending on the time of the year. These variations will now be examined more closely in sections which will deal successively with (a) fertilizations occurring each month, (b) the embryos of intermediate development, and (c) the embryos of maximum size found in the parent at different times of the year.

Fertilizations occurring each month

In the present study, embryos ranging from zygotes to the earlier phases of "fetal larval" development are considered to represent the products of fertilizations most likely to have occurred during the month since the last previous collection. Okada (1936) uses the term "fetal larva" to describe the embryonic stage that commences immediately after gastrulation in *Sphaerium japonicum*. Young in similar stages of development can be found in *Sphaerium simile* (Fig. 6). In this species a comparatively early fetal larva is spherical, pear-shaped or top-shaped, and its overall outline bears no resemblance to that of a typical clam. Cilia can be seen on some of the external surfaces of the embryo. Internally, although various cell masses represent the primordia of future organs, the only clearly recognizable structure is a simple digestive tube, ciliated in part. Unfortunately, because no information is available concerning the rate of embryonic development in *Sphaerium*, it is impossible to determine exactly when the fertilizations occur that produce the earliest developmental stages found each month, especially the early "fetal larvae". Nevertheless, these embryos are still very small and primitive in structure, representing a stage that begins to develop just after gastrulation; it seems likely, therefore, that these young are less than 1 month old.

Approximately 50% of the specimens (of all sizes) examined histologically in September and about 40% of the clams studied in this manner each month from October to April contain stages that are either early fetal larvae or, much less commonly, younger embryos, resulting from even more recent fertilizations (*i.e.*, blastula or gastrula stages). Zygotes and cleavage stages are never seen. Specimens examined in May show no evidence of any stages earlier than early fetal larvae and only one clam contains these. Beginning in June and continuing throughout the summer months, there is considerable evidence of fertilization. Stages of development ranging from zygotes to the earlier phases of fetal larval development, which are almost non-existent in May, are found in 67% of the June specimens, 80% of the July specimens and over 70% of the August specimens examined. These are the only months of the year when zygotes and cleavage stages can be found. Embryos in the blastula and gastrula stages, which were very infrequently encountered in previous months, are commonly seen throughout the summer.

In general it appears that fertilization continues to some extent throughout the year. This activity is at a particularly low level in May, but it shows a marked increase during the summer months.

Intermediate embryos

Although not all the adults examined each month contain embryos less than 1 month old or young almost ready to be born, practically all specimens over 12 mm long are always maintaining embryos in various stages of development between these 2 extremes. For the purposes of the present discussion, such young will be termed "intermediate" embryos. Embryos included in this category range in development from embryos approximately 0.3 mm long to young which are slightly over 3 mm in length. The smallest intermediate embryos have a distinct "clam-like" outline due to the presence of a definite foot and small mantle and gill buds

TABLE I

Seasonal variations in numbers of intermediate young in clams over 14 mm in length

Number of intermediate young	Winter (Dec.-Mar. 2)	Spring (Mar. 21-May)	Summer (June 22-Aug.)	Fall (Sept.-Nov.)
2	0.0%	0.0%	17.6%	0.0%
3	6.7%	0.0%	17.6%	25.0%
4	33.3%	35.3%	52.8%	16.3%
5	20.0%	23.5%	6.0%	16.3%
6	40.0%	41.2%	6.0%	42.4%
Number of adults examined	15	17	17	12

(Fig. 7). Since they appear to be considerably more advanced in both internal and external development than early fetal larvae, it is quite probable that these more well developed embryos are greater than 1 month old. Only specimens exceeding 14 mm in length show definite seasonal variations in the number of intermediate young they contain; therefore, the intermediate embryos of only those adults longer than 14 mm will be considered in the section that follows.

The figures in Table I show that during the winter and spring months most of the large specimens contain between 4 and 6 intermediate embryos. These represent the results of at least 2 or 3 distinct fertilization periods. It is interesting to note that although an active birth period occurs during the winter, while fewer young are released in the spring, the variations in the number of intermediate embryos found in individuals collected during the 2 seasons are surprisingly small. Apparently, fertilizations occurring throughout the winter and early spring supply new young to replace those released, and these embryos grow and develop during this period, gradually entering successively more advanced phases of intermediate development.

As shown in Table I, during the summer months the majority of adults over 14 mm in length contain between 2 and 4 intermediate embryos. There are several factors which could account for this phenomenon. Since fertilization appears to occur at a very low rate between late April and late May, relatively few embryos would be produced which might attain intermediate status by June. Indeed, 4 out of 6 of the adults over 14 mm in length examined at this time are devoid of the smallest intermediate embryos, although they are commonly found in adults of this size in other months of the year. Furthermore, during the summer, when birth is intense, many of the more advanced intermediate embryos continue their growth and development until they are ready for release into the outside environment. Finally, the adults themselves may be undergoing a particularly rapid burst of growth during the summer, and it is possible that smaller adults could enter a larger size category without a concomitant increase in the number of intermediate young they possess.

The continuation of fertilization activity throughout the summer and fall and the declining rate of birth in the autumn enable the majority of specimens longer than 14 mm to accumulate between 4 and 6 intermediate young during the fall months.

Embryos of maximum size in the parent and the percentage of "newborn" young in the population

As is true of the other embryos in a single monthly collection, the oldest young may vary considerably in size and development even among parents of approximately the same length. Nevertheless, both the relative size of the largest embryos in the parent and the percentage of newly released young in the population do show seasonal variations, and together these provide a good indication of when birth most commonly occurs in *Sphaerium simile*. Both of these factors will now be examined. Since clams less than 13.5 mm in length rarely, if ever, contain young which are ready for release, only the embryos of those adults larger than 13.5 mm will be considered.

The data pertinent to this section are summarized in Table II and Figure 9. It should be emphasized that in collecting the data for Table II, only the largest embryo being brooded in each inner gill of the parent was considered; even if smaller young were present, information concerning their sizes was *not* included in this table. In most collections some adults could be found containing, as their young of maximum size, embryos less than 1 mm in length. It was very difficult to obtain accurate measurements of these young with the methods available; therefore, although the percentage of adults containing such embryos is indicated in Table II, young less than 1 mm in length are not included in the data concerning the average size of the largest young. With regard to Table II it should also be noted that histological examination shows that embryos greater than 3.5 mm in length are fully developed. They resemble the adult in every respect other than size and the fact that the gonads are not yet producing gametes (Fig. 8). From these observations, it is concluded that embryos of this size are very large young near birth.

Clams ranging from 5 mm to just under 8 mm in length represent the smallest individuals occurring in the population. Since embryos of 6 mm or 7 mm in length

TABLE II

Embryos of maximum size in adults over 13.5 mm in length

	Percentage of adults whose embryos of maximum size are:			Average size of largest embryos over 1 mm long	Total number of adults examined
	Less than 1 mm long	1 mm–3.5 mm long	over 3.5 mm long		
Nov.	0.0%	57.2%	42.8%	3.5 mm	7
Dec.	no specimens examined				0
Jan.	6.3%	87.4%	6.3%	2.0 mm	16
Feb.	41.7%	41.7%	16.6%	2.5 mm	12
Mar.	5.6%	72.2%	22.2%	2.5 mm	18
Apr.	0.0%	71.4%	28.6%	2.8 mm	22
May	0.0%	40.0%	60.0%	3.9 mm	20
June	25.0%	37.5%	37.5%	3.7 mm	24
July	9.1%	72.7%	18.2%	2.4 mm	22
Aug.	41.7%	25.0%	33.3%	3.6 mm	24
Sept.	22.7%	63.6%	13.7%	2.4 mm	22
Oct.	9.1%	68.2%	22.7%	2.7 mm	22

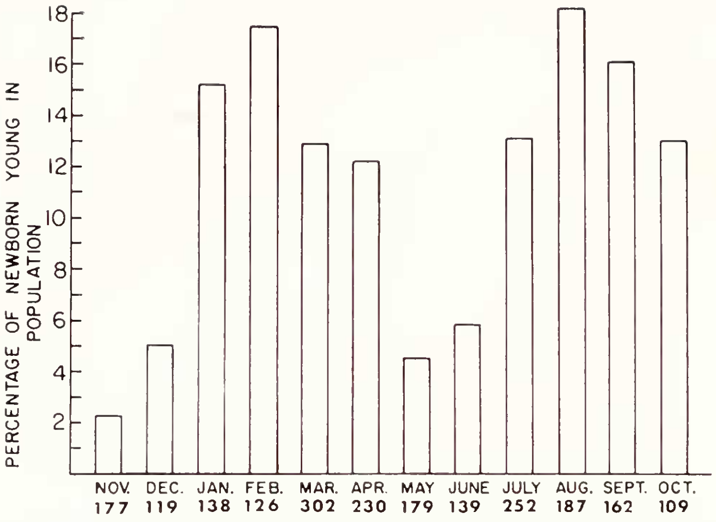

FIGURE 9. Monthly variations in the percentage of "newborn" young in the population. The number under each month indicates the total number of individuals (ranging in length from 5 mm to over 18 or 19 mm) collected that month.

are found inside adults, it is assumed that clams of comparable length in the outside environment represent "newborn" young. Figure 9 shows the percentage of such newborn young, 5 mm to just under 8 mm long in each of the monthly samples.

Figure 9 indicates that although newborn young can be found in the population throughout the year, the 2 major periods of birth for *S. simile* appear to occur during the winter and summer months. Between December and January, the percentage of newly released young in the sampled population shows a threefold increase, and there is a further increase during the following month. The second great burst of birth activity begins to occur between June and July and reaches its peak in August, although a comparatively large percentage of newborn young can still be found in the sample population collected in September. During the spring and fall months, on the other hand, the percentage of young between 5 mm and just under 8 mm long in the sampled populations steadily decreases. It thus appears that these 2 seasons are periods of declining birth activity.

The size of the largest embryos being brooded by the parents also shows seasonal variations, although these are not as clearly defined as the patterns of birth. In both the spring and fall months, when fewer young are released, the average size of the largest embryos in the parent gradually increases in preparation for the next major period of birth. It should also be noted that in those months when the percentage of newly released young in the sampled population is at a particularly low level (*i.e.*, November and December, May and June), the average size of the largest embryos in the parent is, for the most part, considerably greater than it is at other times of the year (Table II). In addition, adults maintaining embryos over 3.5 mm long are also very common at these times. Although the largest embryos of only 2 specimens were examined in December, in both cases the young are between 3 mm and 5 mm in length. In addition, over four-fifths of the adults studied histologically in November and December contain embryos nearly ready to be born. In summary, both the histological observations made in November and December as well as the data in Table II indicate that a large percentage of the adults examined in the months immediately preceding a period of high birth activity contain embryos nearly ready to be born.

In general, during the months when many newborn young are found in the outside population, the largest embryos being brooded by the adults are considerably smaller than they are when birth is at a low level. There is a sharp drop in the average size of the largest embryos in the parents between December and January, June and July, and August and September. It appears that the average size of these embryos rises somewhat between January and "February" (*i.e.*, March 2) and is very high in August, despite the fact that a large percentage of newborn young are found in the outside population in both these months. Nevertheless, it must also be noted that in both "February" and August over 40% of the adults examined contain, as their young of maximum size, embryos less than 1 mm long, whose measurements are not included in the average sizes listed in Table II. Apparently during these months, and especially in August, a number of adults still contain young nearly ready to be born, while many others have released young very recently, and the embryos remaining inside them must undergo considerably more growth and development before they will be ready for birth.

Discussion

The successive phases of reproduction including the production of gametes, fertilization of eggs, maintenance of embryos and birth of young occur to a limited degree at all times. Nevertheless, each activity varies considerably in extent depending on the month or season being considered, and it is these seasonal variations that produce the annual reproductive cycle characteristic of *Sphaerium simile*.

Both gametogenesis and fertilization occur throughout the year. The fact that mature gametes are not present in large numbers from September to April probably accounts for the fact that less than half the individuals examined in each of these collections contain embryos resulting from very recent fertilizations. The testes of all specimens examined in both April and May generally contain very few spermatozoa, and this fact helps to explain the particularly low level of fertilization observed in May. During the period from June to August, when mature gametes of both sexes are more common than they are in other months, fertilization shows a marked increase.

Young in several stages of development can be found in parent clams throughout the year. During both the spring and the fall months the largest embryos of adults over 13.5 mm in length gradually increase in size, and these young are born mainly during the winter and summer months. In general, individuals of specified length show surprisingly minor variation in the number of young they contain, regardless of the season. It thus appears that a continuous succession of young is maintained in parent clams. When the oldest embryos are released, the other groups of young in the parent continue their development, and ultimately a new group is ready for release. Meanwhile the replacement cycle continues as new groups of eggs undergo fertilization.

In many species of the genera *Sphaerium* and *Musculium* each phase of reproduction occurs in several seasons of the year. Although Drew (1896) and Gilmore (1917) did not make any attempt to study seasonal changes in the gonads of *S. simile,* both investigators observed mature gametes throughout the year in this species. A similar phenomenon is observed in *S. striatinum* (Woods, 1931). Okada (1935b) noted that in *S. japonicum* the presence of mature gametes and zygotes is limited to the spring and fall.

With regard to the release of young, Okada (1935b) noted that in *S. japonicum* birth occurs most commonly during the spring and autumn. Investigations on the life cycle of *S. corneum* carried out by Thiel (cited in Okada, 1936b) and more recently by Mitropol'skii (1969) indicate that in this species birth is most frequent in the summer and fall, although Mitropol'skii found no sharp division between these 2 birth periods. Foster (1932), working with *S. striatinum,* reported that in this species birth occurs mainly during the summer and winter, and the present study shows that the situation is similar in *S. simile.* The main difference between the times of birth in these 2 species is that in *S. striatinum* these periods are more sharply defined than they are in *S. simile.* In *S. striatinum* many young are released in the winter, and then birth is minimal until July when births increase until August. In *S. simile* birth continues at a high rate throughout the winter and summer months and gradually declines during the spring and fall. The differences in birth cycles in these 2 species may be partly explained on the basis of the numbers of embryos that each is capable of maintaining. Although Avolizi (1971) observed that *S. striatinum* may contain as many as 12 embryos, Foster (1932) reported that most of the adults in the population of *S. striatinum* that he observed contain 2 to 4 developing young. In the population of *S. simile* investigated in the present study there are normally 6 to 8 developing young. It may be that it simply takes longer for the larger number of young produced in *S. simile* to be released into the general population, and this may lead to the larger overall birth periods, tapering off gradually, observed in this species.

With regard to litter size in *Sphaerium simile,* Gilmore (1917) reported that no more than 2 to 4 embryos are found in the inner gill of this species. The discrepancy between Gilmore's observations and those of the present study may be explained by the fact that Gilmore based his conclusions concerning brood size primarily on data obtained from a number of gross dissections of clams examined in July 1917. With the technique used he was unable to observe embryos less than 0.5 mm in length, and smaller young were therefore not included in the results of this part of his study. In the present investigation, it was observed that in July many of the largest young are released from the parent by birth, and in addition, many new eggs are fertilized. In the majority of specimens examined, at least half the embryos they contain are considerably less than 0.5 mm long and are often not visible without the aid of a microscope. Thus it seems probable that the methods Gilmore employed were not adequate to determine the total number of young being brooded by *S. simile.*

In some sphaeriid populations reproductive activities are limited to those seasons of the year when environmental conditions are most favorable. Thomas (1963) studied a population of *Musculium partumeium* living in a temporary pond which freezes in the winter and dries up during the summer. Both growth of the individuals and the release of young are confined to the spring months when the amount of water in the pond and its temperature provide suitable conditions for these activities. The habitat of the population of *S. simile* investigated in the present study does not undergo extreme environmental changes. Sphaerium Brook never dries up, and a steady moderate current is present throughout the year. This flow of water prevents the brook from freezing; even during the intensely cold weather in the winter of 1969–1970 water temperatures of 2° C to 3° C were measured, and ice formed only along the banks. In late summer, although water temperatures as high as 26° C were noted, conditions favorable for reproduction are maintained through the steady movement of oxygenated water from the pond. This comparatively mild environment permits reproductive activities to continue throughout the year in *S. simile*.

Both Foster (1932) and Thomas (1965) reported that the total life span of the species they investigated was approximately 1 year. Herrington (1948), on the other hand, working with *S. occidentale*, noted from marking experiments that this species can survive longer than 1 year. The populations of *S. striatinum* investigated by Avolizi (1971) have a life span of 18 to 24 months. Avolizi's study also indicates that *S. simile* survives approximately 26 months and has a steady but low rate of mortality.

The present study has indicated the annual reproductive cycle of *Sphaerium simile*. It is hoped that this investigation together with Avolizi's work concerning the growth rate and longevity of this species will provide a more comprehensive understanding of the entire life cycle of *Sphaerium simile*.

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Summary

1. Reproduction occurs throughout the year in *S. simile* although definite variations can be observed in different reproductive activities at different times.

2. Gametogenesis and mature gametes are visible to some extent during the fall, winter and early spring (particularly in specimens less than 12 mm long); but the production of gametes appears to be most intense in all adults, regardless of size, in the late spring and summer (especially June and July).

3. Fertilization seems to be most active during the summer, although parents with young resulting from very recent fertilizations can be found throughout the year.

4. Embryos in one or more stages of development are present in the great majority of individuals over 10 mm in length at all times.

5. The birth rate is highest during the winter and summer months. There is a gradual decline in the number of births throughout the spring and fall. During these seasons, however, the embryos inside the parent continue their growth and

development; the size of the largest embryos in the adults continues to increase in preparation for the next major period of birth.

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